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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P2051PC00	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/DK2005/000126	International filing date *(day/month/year)* 24.02.2005	Priority date *(day/month/year)* 24.02.2004
International Patent Classification (IPC) or national classification and IPC INV. C12N1/00 C12N1/04		
Applicant CHR. HANSEN A/S et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 4 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21.12.2005	16.05.2006
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Stoyanov, B Telephone No. +49 89 2399-7726

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/DK2005/000126

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-26 as originally filed

   Claims, Numbers

   1-13 received on 29.12.2005 with letter of 21.12.2005

   Drawings, Sheets

   1/2, 2/2 as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-13
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	1-13
	No:	Claims	-
Industrial applicability (IA)	Yes:	Claims	1-13
	No:	Claims	-

2. Citations and explanations (Rule 70.7):

see separate sheet

Section V

The combination of pellet frozen lactic acid bacteria and the additives of claim 1 was not known from the prior art. The present international application provides the unexpected technical effect of increasing the Tm value of the pellets and keeping them free flowing whilst frozen. Correspondingly, present application is deemed to comply with the requirements of Art. 33(2)(3) PCT.

For the sake of completeness it is noted that present claim 6 has an incorrect dependancy.

It is also noted that present claim 12 is superfluous.

CLAIMS

1. A pellet-frozen lactic acid bacteria (LAB) culture in a commercially relevant package that has a weight of at least 50 g frozen material, wherein the frozen material is present in the form of individual pellets, having a content of viable bacteria of at least 10^9 colony forming units (CFU) per g frozen material and comprising from 0.5% to 13% of an additive compound measured as w/w of the frozen material, wherein the additive compound is an additive compound that is selected from the group of additive compounds consisting of Cyclodextrin, Maltitol, Trehalose, Fish gelatin, Maltodextrine, Yeast Extract and Spray gum, and which further is characterized by,

when using an amount of 10% of the additive compound measured as w/w of the frozen material, the compound is able to increase the Tm' (onset temperature of ice melting) of the frozen lactic acid bacteria (LAB) culture, which without the additive compound has a Tm' value from -70°C to -46°C, to a Tm' value above -46°C, such as from -45°C to -15°C (measured by DSC)

and wherein the frozen lactic acid bacteria (LAB) culture is characterized by that when stored at approximately -46°C for 7-14 days the individual pellets of the frozen culture are not sticking together and therefore substantially remain as individual pellets where this is measured by following test

the individual pellets of the frozen culture are pellet frozen in liquid nitrogen and 100 individual pellets (around 5 – 100 g of pellets) are poured into a petridish, thus forming a thin layer of loose individual single pellets, the layer being characterized in that the majority of the pellets are in physically contact with one or more of its neighbor pellets, placed at approximately -46°C for 7-14 days and examined to see if the pellets are still loose or if the pellets had made clumps or are sticking together wherein the criteria for that the individual pellets of the frozen culture substantially remain as individual pellets are that at least 80 of the 100 individual pellets remain as loose individual single pellets;

with the exception of a frozen lactic acid bacteria (LAB) culture that comprises LAB that are able to utilize sucrose and wherein the culture comprises cryoprotective agent compound selected from the group consisting of sucrose in an amount from 2 % to 13 % of sucrose measured as w/w of the frozen material; and trehalose in an amount from 4 % to 6 % of trehalose measured as w/w of the frozen material; and a trehalose/sucrose mixture both in the amount of 13% measured as w/w of the frozen material.

2. The pellet-frozen culture of claim 1, wherein the culture is a mixed mesophilic culture consisting of mesophilic bacteria having optimum growth temperatures at about 30°C.

3. The pellet-frozen culture of claim 1 or 2, wherein the LAB is a LAB selected from the group comprising Bifidobacterium spp., Brevibacterium spp., Propionibacterium spp., Lactococcus spp. including Lactococcus lactis subsp. lactis and Lactococcus lactis subsp. cremoris, Lactobacillus spp. including Lactobacillus acidophilus, Streptococcus spp., Enterococcus spp., Pediococcus spp., Oenococcus spp. and fungal spp. including Pencillium spp., Cryptococcus spp., Debraryomyces spp., Klyveromyces spp. and Saccharomyces spp.

4. The pellet-frozen culture of any of the preceding claims, wherein the frozen lactic acid bacteria (LAB) culture is a culture which without comprising the additive compound according to claim 1 has a Tm' value of from -70°C to -46°C.

5. The pellet-frozen culture of any of the preceding claims, wherein the frozen lactic acid bacteria culture comprises from 5% to 10% of the additive compound measured as w/w of the frozen material.

6. A method for making a pellet-frozen lactic acid bacteria (LAB) culture of any of the claims 1 to 6 comprising the following steps:

(i) adding an additive compound to viable bacteria to get at least 50 g of material with a content of viable bacteria of at least 10^9 colony forming units (CFU) per g material and comprising the additive compound in an amount from 0.5% to 13% measured as w/w of the material,

(ii) freezing the material to get pellet-frozen material, and

(iii) packing the pellet-frozen material in a suitable way to get a packed frozen lactic acid bacteria (LAB) culture of any of the claims 1 to 6.

7. The method of claim 6, wherein

before adding the additive compound according to step (i) of claim 6 one has measured the Tm' value of the frozen lactic acid bacteria (LAB) culture without comprising the additive compound and identified that it has a Tm' value of from -70°C to -46°C;

and

after adding the additive compound is the Tm' value of the frozen lactic acid bacteria (LAB) culture comprising the additive compound measured and it is verified that the Tm' value is from -49°C to -15°C, more preferably from -39°C to -15°C.

8. The method of claim 6 or 7, wherein the culture is a mixed mesophilic culture consisting of mesophilic bacteria having optimum growth temperatures at about 30°C.

9. The method of claim 6 to 8, wherein the LAB is a LAB selected from the group comprising Bifidobacterium spp., Brevibacterium spp., Propionibacterium spp., Lactococcus spp. including Lactococcus lactis subsp. lactis and Lactococcus lactis subsp. cremoris, Lactobacillus spp. including Lactobacillus acidophilus, Streptococcus spp., Enterococcus spp., Pediococcus spp., Oenococcus spp. and fungal spp. including Pencillium spp., Cryptococcus spp., Debraryomyces spp., Klyveromyces spp. and Saccharomyces spp.

10. The method of claim 6 to 9, wherein the frozen lactic acid bacteria culture comprises from 5% to 10% of the additive compound measured as w/w of the frozen material.

11. The method of claim 6 to 10, wherein the additive compound is an additive compound selected from the group consisting of Cyclodextrin, Maltitol, Trehalose, Fish gelatin, Maltodextrine, Yeast Extract and Spray gum.

12. A pellet-frozen lactic acid bacteria (LAB) culture obtainable by the method for making a frozen lactic acid bacteria (LAB) culture of claim 6 to 11.

13. Use of the pellet-frozen lactic acid bacteria (LAB) culture of any of claims 1-5 and 12 in a process for making a food or feed product.